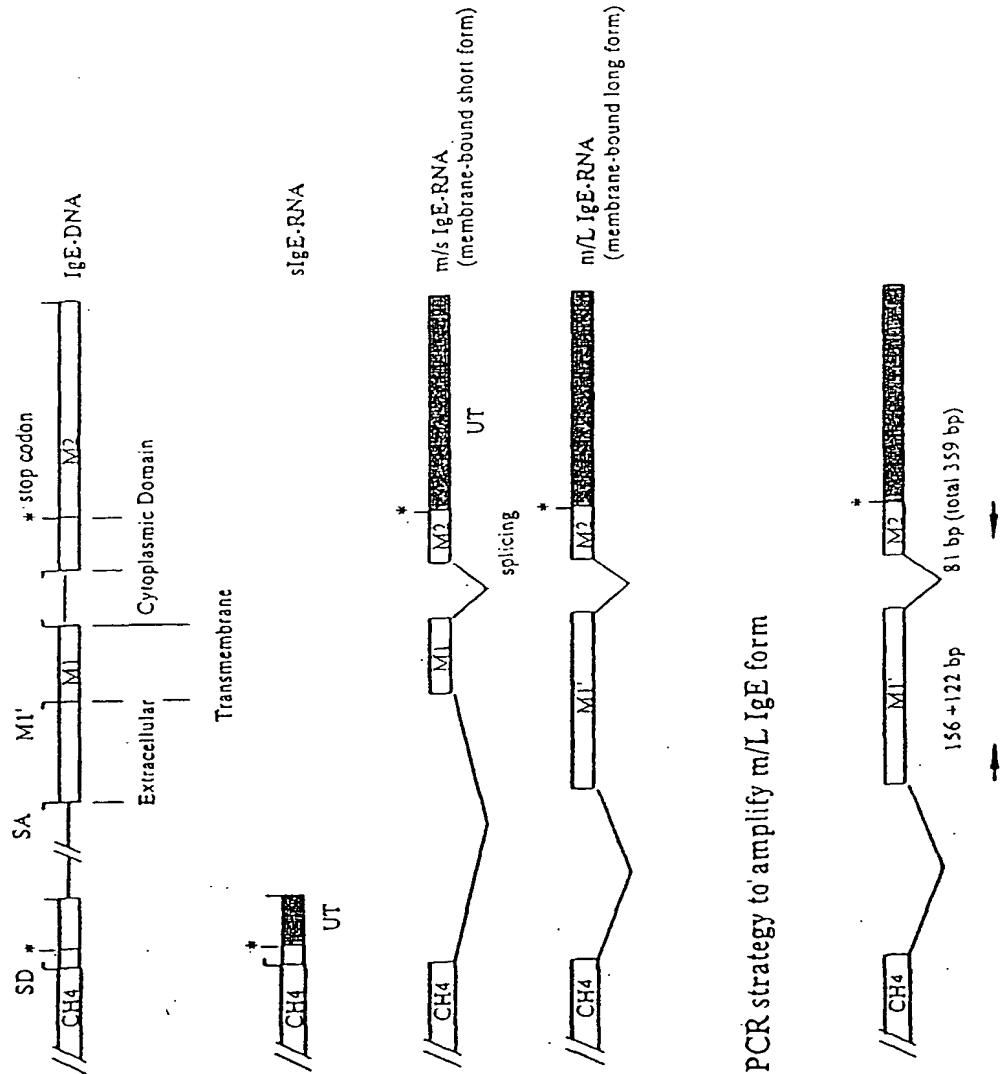


# Construction of membrane-bound IgE

## I. Cloning of human membrane-bound IgE gene by RT-PCR

A. Schematic representation of the secretory and two membrane human IgE H chain isoforms



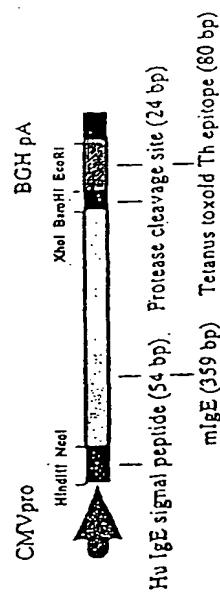
B. RT-PCR strategy to amplify m/L IgE form

Fig.1

# Construction of membrane-bound IgE

## II. Construction of a vector insert for expression of a mIgE fused to TT

### A. Human mIgE - Tetanus toxoid fusion protein expression cassette



### B. PCR amplification with specific primer set

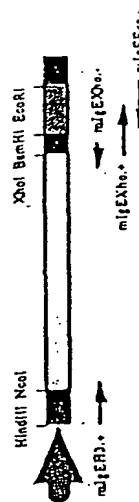


Fig.2

# In Vitro Expression of mIgE Construct I.

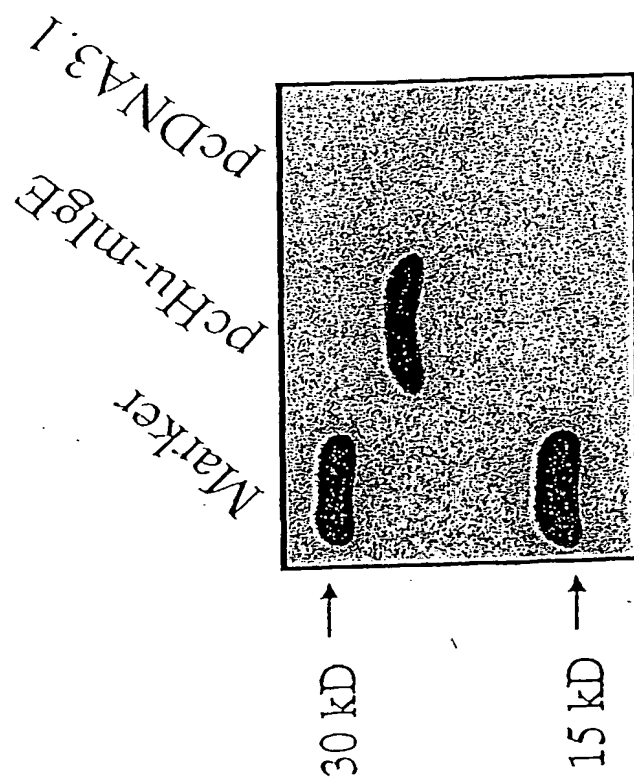


Fig.3

# In Vitro Expression of mIgE Construct II.

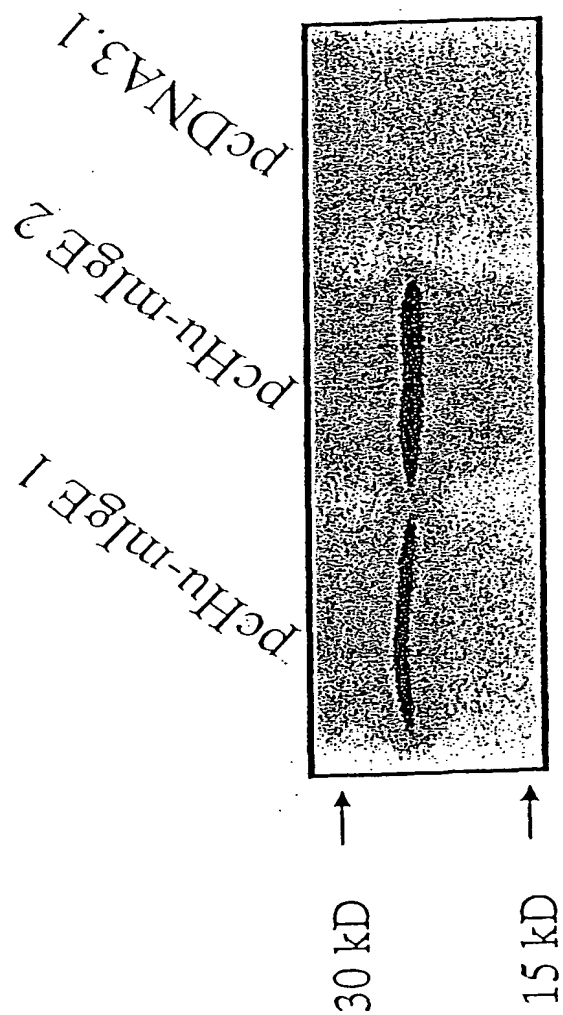


Fig.4

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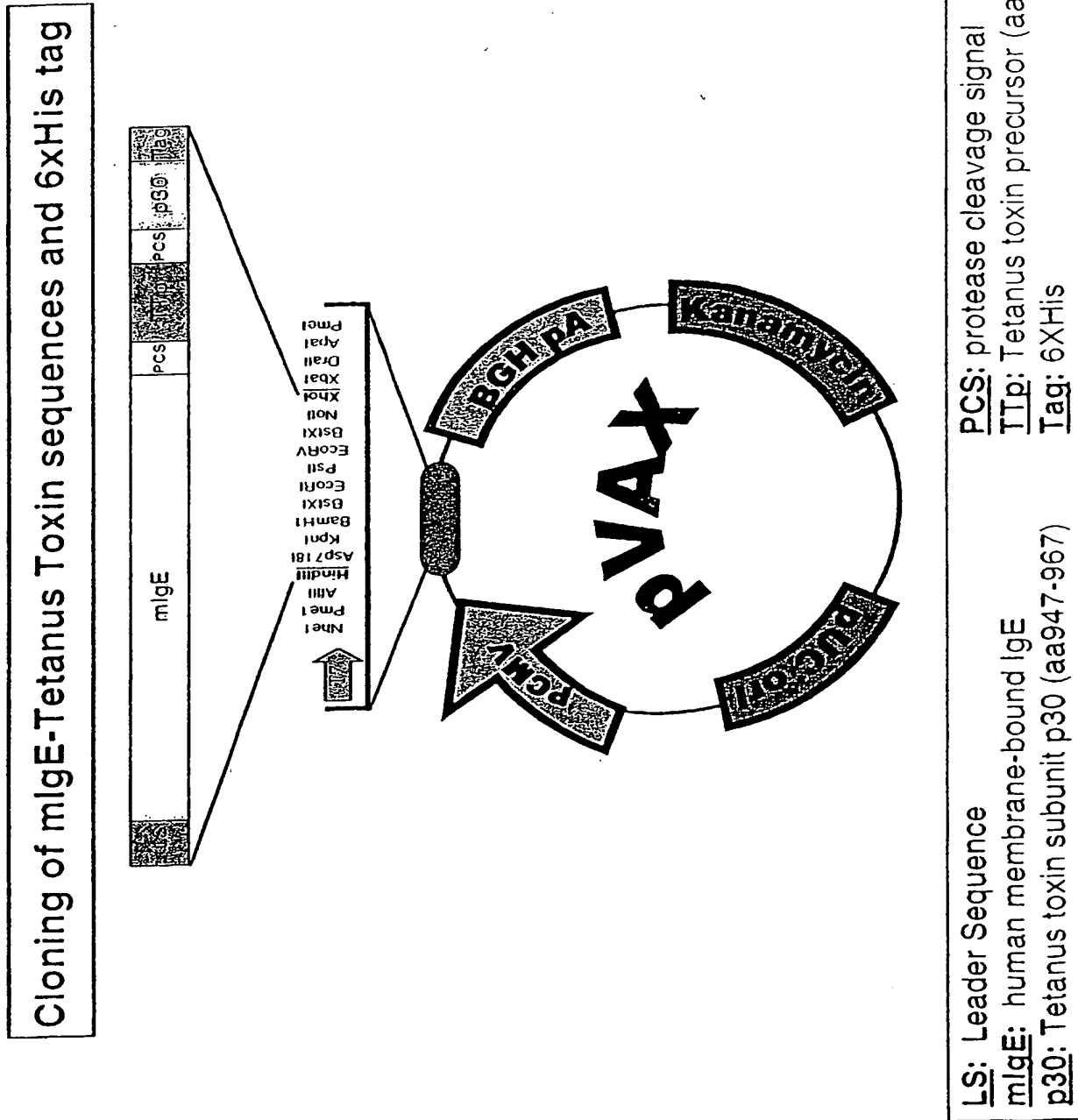


Fig.5

**mlgE-TT construct: Nucleotide and amino acid sequences**

(SEQ ID NOS: 5 &amp; 6)

atggactggacctggatcctcttcttgggtggcagcagccacgcgagtcactcccatggg

M D W T W I L F L V A A A T R V H S | H G

Leader sequence

ctggctggcggctccgcgcagtcaccagagggcccgga taggggtgctctgccactccgga

L A G G S A Q S Q R A P D R V L C H S G

mlgE

cagcagcagggactgccgagagcagcaggaggctctgtccccacccccgctgccactgt

Q Q Q G L P R A A G G S V P H P R C H C

mlgE

ggagccgggagggctgactggccaggtccccagagctggacgtgtgcgtggaggaggcc

G A G R A D W P G P P E L D V C V E E A

mlgE

gagggcgaggcgccgtggacgtggaccggcctctgcactcttcgcgcactcttctctgtc

E G E A P W T W T G L C I F A A L F L L

mlgE

agcgtgagctacagcgccgcctcacgctcctcatgggtgcagcggttcctctcagccacg

S V S Y S A A L T L L M V Q R F L S A T

mlgE

cggcaggggaggccccagacctccctcgactacaccaacgtcctccagccccacgccaga

R Q G R P Q T S L D Y T N V L Q P H A | R

mlgE

gaaaaaagagctgttggttggttacgatccaaattatttaaggactgattctgataaagat

E K R A V V G Y D P N Y L R T D S D K D

Protease cleavage signal

TTp

agatttttacaaaccatgggtaaaactgtttaacagaattaagagagaaaaaagagctgtt

R F L Q T M V K L F N R I K R E K R A V

TTp

Protease cleavage

gttggttttaataattttaccggttagcttttggttgagggttcctaaagtatctgctagt

V G F N N F T V S F W L R V P K V S A S

signal

p30TT

catttagaacatcatcatcatcattag

H L E H H H H H H -

Flag

**Fig.6**

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## Synthetic peptides used as antigens for in vitro assays

Name	Amino acid sequence	Human mIgE Region
IgEEx#1	(SEQ ID NO: 7) SAQSQRAPDRVLCHSGQQQLP	extracellular (22 aa)
IgEEx#2	(SEQ ID NO: 8) AGGSVPHPRCHCGAGRADWPGP	extracellular (22 aa)
Migis	(SEQ ID NO: 9) ELDVCEEEAEGEAPW	extracellular (15 aa)
CTL2	(SEQ ID NO: 10) EAPWTWTGL	extracellular-transmembrane (9 aa)
CTL1	(SEQ ID NO: 11) TGLCIFAALF	transmembrane (9 aa)
IgECyt	(SEQ ID NO: 12) VQRFLSATRQGRPQTSLDYTNVLQPHA	intracellular (27 aa)
TTh (27 aa)	(SEQ ID NO: 13) YDPNYLRTDSDKDRFLQTMVKLFNRIK	

Fig. 7

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IFN- $\gamma$  production determined in splenocytes from immunized mice  
(7 days after 3rd immunization) after stimulation with IgE peptide antigen

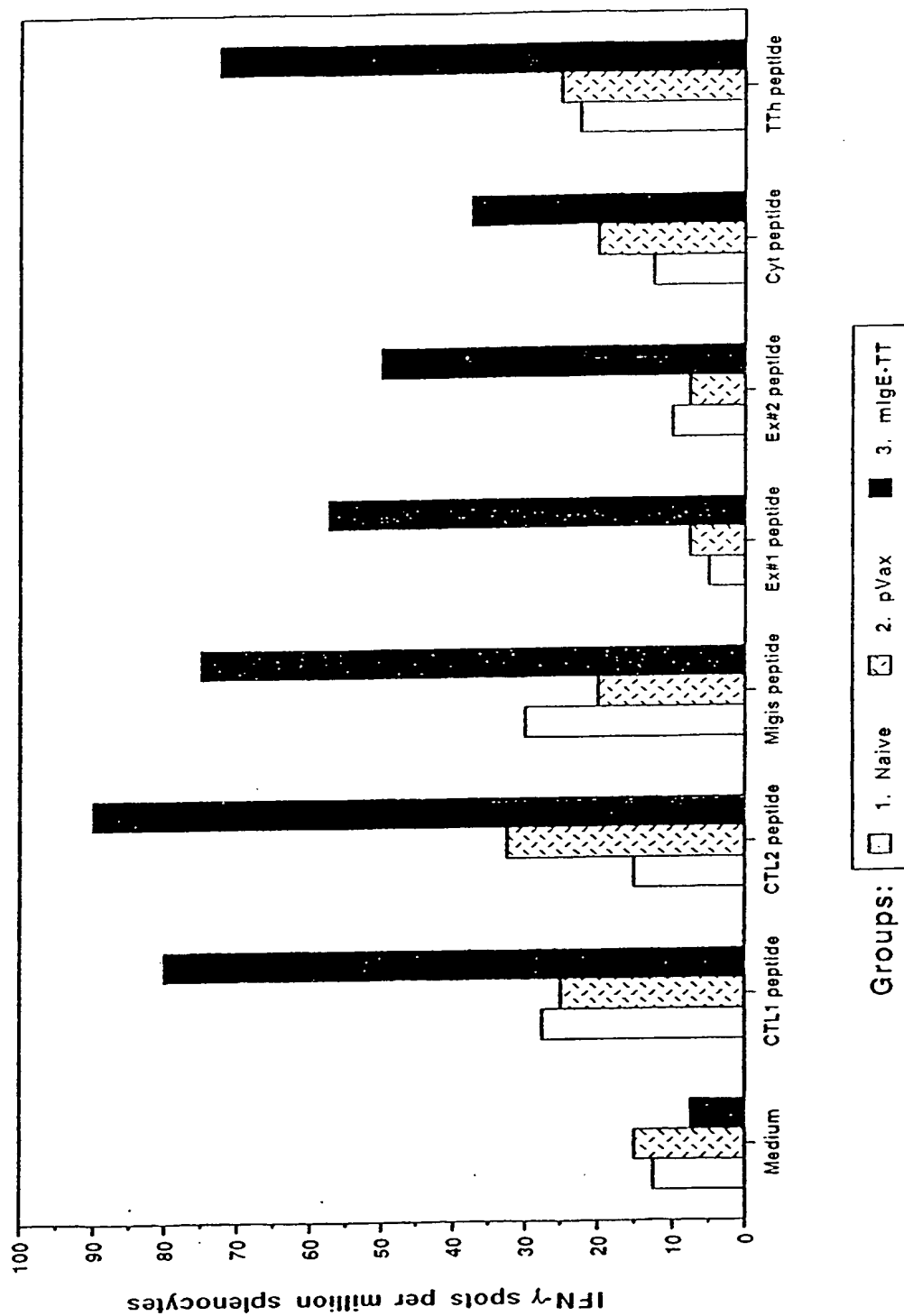


Fig.8